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A PRELIMINARY EXPERIMENT OF REJUVENATION IN *Turritopsis* sp. (HYDROZOA, ANTHOMEDUSAE) BY FRESHWATER IMMERSION

By

Kazuhito NIINA¹ and Shin KUBOTA²**Abstract**

Immature medusae of *Turritopsis* sp. obtained from cultured polyps originated from a medusa collected from Shirahama, Wakayama Prefecture, Japan at the Seto Marine Biological Laboratory, Kyoto University were experimentally treated 50 % seawater (16 psu) or 100 % freshwater (DW) immersion for the following minutes: 5, 10, 120. Only one medusa can be rejuvenated unexpectedly due to unknown reason, keeping it in the 50% seawater for 120 mins. We infer that instant immersion caused sudden drop of salinity is unfavourable and severe even in 5 minutes immersion, resulting very low rate of rejuvenation.

Introduction

Turritopsis spp. (Hydrozoa, Anthomedusae) are known that they can be rejuvenated by aging or stress. It has been demonstrated by experiments that when *Turritopsis* has had severe damage, they have been rejuvenated (Kubota 2005; 2011; 2013). When salinity of the sea water in all of the aquariums at the laboratory dropped caused by typhoon No. 18 on 15th September 2013, we found incidentally that a *Turritopsis* medusa was rejuvenated by the stress of the low density of salt (Kubota and Niina 2014). Thereafter this rejuvenated polyp produces many medusae and some of them can be continuously rejuvenated. Based on this fortuitous event, we proceeded with a preliminary experiment to check if the *Turritopsis* medusa can be rejuvenated in artificially lowered salinity of seawater or freshwater. We present such a result in this report.

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Materials and methods

We have bred a polyp colony that was rejuvenated from an immature medusa that was collected in Tanabe Bay, Shirahama Town, Wakayama Prefecture in September, 2010 (Kubota 2013). We kept it at around 26°C throughout the year by using a heating system during the winter (Kubota and Niina 2016). On 11th and 14th January, and on 23rd November, 2014, we did the following exercises, using 18 individuals of very young medusae that had separated from this colony just a few days before our commencement of the experiment. We prepared 50% density of sea water and 100% of freshwater (DW), and we kept six groups of three medusae individually in water for 5 mins/10 mins/120 mins, then studied the progression. To study all these progress, we kept these medusae in the natural sea water that was filtered by a 5 μ m sieve (Marfied, USA) and maintained the water temperature at 26°C. No control experiment using natural sea water is done since rejuvenation has been taken place in the same colony many times before.

Results and discussion

Contrary to our expectation, out of all experiments using a total of 18 immature medusae, only once rejuvenated as our surprisingly result due to unknown reason, i.e. keeping one medusa in the 50% seawater for 120 mins on 14th January, 2014 (Table 1). From these experiments, we presume that one of the reasons for bringing about extremely very rare case of rejuvenation is that they were not able to adjust themselves to the new experimental conditions sufficiently because they were transferred instantly into the low salinity (0 or 50%).

Table 1. Number of individuals rejuvenated from immature *Turritopsis* medusa to polyp.

Medium	Time of immersion (min.)		
	5	10	120
DW	0 (3)*	0 (3)*	0 (3)*
Diluted SW** (16psu)	0 (3)*	0 (3)*	1 (3)*

*: No. of individuals examined.

** : Natural seawater by a 5 μ m sieve

A big difference between the typhoon case in 2013 (Kubota and Niina 2014) and the present case is, we infer, the rain caused by the typhoon made the circulated sea water drop its density gradually, not instantly. But 2015 typhoon effect after change of duct system of our

laboratory since the summer of 2015 seems to be affected survival of polyp life and all the polyps died unfortunately (Kubota and Niina 2016). Further experiments are required to set the same species in different environmental conditions such as lowering the density of seawater gradually and/or more various diluted density of seawater, since 50 % dilution is too severe and unfavourable as freshwater (DW).

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